9. ANALYTICAL PROCEDURES

STANDARD 9.1

The WCSO DNA Procedure Manual and the CODIS Procedure Manual approved by the Technical Leader detail the analytical procedures utilized by the WCSO DNA Section. The standard operating procedures are reviewed annually by the Technical Leader independent of the audit required by Standard 15 and documented through revisions.

The only controlled form that the DNA Section utilizes is the Technical Review Form (Doc ID 1797); all other forms are uncontrolled.

STANDARD 9.2

The WCSO DNA Section uses reagents that are suitable for the methods employed.

9.2.1 All materials, reagents, chemicals, and supplies will be received into the DNA section and inventoried against the order. The initialed and dated packing slip will be forwarded to the front office staff or to the appropriate procard user/purchaser if purchased on a procard.

If the DNA analyst finds that a particular supply, chemical reagent, or material does NOT meet the required quality control standards, he / she shall immediately notify the Technical Leader. The manufacturer will be contacted and the reagent will be returned to the manufacturer. If a hazardous chemical has spilled during shipping, the MSDS and appropriate protocol in the Division Safety Manual will be followed.

The lot number of component sources used to prepare reagents, e.g. Buffer X1, Digest Buffer, and TE^{-4} will be tracked along with the date of preparation and the initials of analyst that prepared the reagent. The lot number for prepared reagents will be the date of preparation, for example, if Digest Buffer was prepared on 03/12/12 then the lot number for the prepared reagent would be 031212.

9.2.2 All reagent labels must include identity, date of preparation, identity of the individual preparing the reagents, the expiration date, and storage requirements; otherwise all reagents are stored according to manufacturer specifications. The following is a list of reagents and their associated expiration dates:

- QIAgen kit reagents: 1 year from receipt. Reagents that are diluted with 200 proof ethyl alcohol will use the expiration date of 200 proof ethyl alcohol if it expires prior to the kit expiration date
- 200 proof ethyl alcohol: 3 years from date of manufacture or as determined by the manufacturer
- DTT: 3 years from the date of receipt (prepared DTT will have the same expiration date as stock)

- Ultra pure 10% SDS: 5 years from date of receipt
- 1M Tris-HCL pH 7.5: 5 years from date of receipt
- 0.5 M EDTA: as determined by the manufacturer
- Sodium Chloride 5 M solution: as determined by the manufacturer
- Digest Buffer: expiration date of component reagent that will expire first
- Buffer X1: expiration date of component reagent that will expire first
- TE^{-4}: expiration date of component reagent that will expire first
- Plexor HY kit: as determined by the manufacturer
- PowerPlex 16 HS kit: as determined by the manufacturer
- GlobalFiler kit: as determined by the manufacturer
- Yfiler kit: as determined by the manufacturer
- GeneScan 500 LIZ size standard: 1 year from the date of receipt
- GeneScan 600 LIZ size standard: as determined by the manufacturer
- HiDi Formamide: 1 year from the date of receipt
- POP-4: as determined by the manufacturer
- Buffer (10x) with EDTA: 1 year from the date of receipt
- Multi-Capillary DS-33 Matrix Standard Kit: 1 year from the date of receipt
- Plexor Calibration kit Set A: as determined by the manufacturer
- PowerPlex Matrix Standards: as determined by the manufacturer
- DS-36 (Dye Set 6) Matrix Standard Kit: as determined by the manufacturer

Due to the nature of the chemicals in Clorox Bleach Germicidal Cleaner and DNA Exitus Plus, these cleaning solutions will not be used past their expiration dates. The expiration date for 70% ethanol will be 10 years from the date of receipt. The expiration date for bleach will be 1 year from the date of receipt.

STANDARD 9.3

The following **CRITICAL REAGENTS** require a quality control (QC) test before use in casework. The QC results will be maintained in a binder.

- Qiagen Extraction Kits and all separately purchased kit components
- Extraction Reagents: Digest Buffer, DTT, and Buffer X1
- Commercial Quantitation Kits
- Commercial Amplification Kits
- TE^{-4}

Each reagent/kit shall be tested using a sample containing known DNA and a reagent blank. The reagents/kits will be deemed as passing the critical reagent test if the amplified DNA types as expected from the known and no DNA is detected upon quantitation and amplification of the reagent blank. If any critical reagent does not pass quality control testing, the manufacturer will be contacted and the reagents will be returned. See each procedure for more details on the quality control testing requirements.

STANDARD 9.4

The WCSO DNA Section procedures include a section on the quantitation of human DNA.

STANDARD 9.5

The WCSO DNA Section monitors the analytical procedures using the following controls and standards:

9.5.1. Quantitation standards

9.5.2 Positive and negative amplification controls associated with samples being typed are amplified concurrently with the samples at all loci and with the same primers as the forensic samples.

9.5.3 A Reagent Blank Control / Multiple Reagent Blank Controls shall be:

9.5.3.1 Extracted concurrently with the associated evidence sample(s);

9.5.3.2 Quantitated, with one of the two reagent blank controls demonstrating the greatest signal being amplified and typed or with both reagent blank controls amplified and typed;

9.5.4 A reagent blank control that is amplified and typed shall be:

9.5.4.1 Amplified utilizing the same primers, thermal cycler model, and concentration conditions as required by the evidence sample with the least amount of DNA;

9.5.4.2 Amplified with each amplification kit utilized;

9.5.4.3 Typed using the same CE 3130 and injection conditions (i.e. injection times and voltage) as the associated evidence sample containing the least amount of DNA.

NOTE: If a sample is re-amplified with the same amplification test kit or system, the template volume is not increased over that of the original reagent blank, and the amplification parameters are not increased to increase sensitivity, then re-amplification of the associated reagent blank is not necessary. Additionally, if no samples in an extraction set are carried past quantitation, then the associated reagent controls do not need to be amplified. If amplification of any of the samples in the extraction set is needed in the future, then the associated reagents controls will be amplified as required by 9.5.4.

9.5.5 Allelic ladders and/or internal size markers for variable number tandem repeat sequence PCR based systems.

9.5.6 Appropriate NIST standard reference material or material traceable to a NIST standard is utilized annually or whenever substantial changes are made to the DNA procedures. The WCSO DNA Section has written guidelines for the interpretation of data within the procedures. The NIST standards reference material will be stored according to manufacturer's specifications. The NIST traceable reference sample will be stored in the freezer in room 140 and will be labeled as such.

9.5.7 When swabbing items of evidence, an associated water control (case specific or day control) must also be prepared. Routine analysis of water controls is not required but may be analyzed at the analyst's discretion. If the water control is processed and gives an "N/A" value upon quantification using Plexor HY, no further analysis is necessary. Only one of the multiple reagent controls associated with an extraction set must be amplified and typed if any sample in the extraction set is amplified. However a reagent control must be amplified with each amplification kit used. This reagent control must be the control demonstrating the greatest signal. If there is insufficient reagent control left to amplify with Yfiler, then the associated samples cannot be amplified with Yfiler.

STANDARD 9.6

The laboratory shall have written guidelines for the interpretation of data.

9.6.1 The WCSO DNA Section verifies that all control results meet the laboratory's interpretation guidelines for all reported results.

9.6.2 For a given population(s), the statistical interpretations are made following the recommendations 4.1, 4.2 or 4.3 as deemed applicable of the National Research Council report entitled "The Evaluation of Forensic DNA Evidence" (1996) and/or court directed method. These calculations are derived from a documented population database appropriate for the calculation.

9.6.3 The WCSO DNA section has and follows a documented procedure for the statistical interpretation of Y-chromosomal results (refer to the DNA Procedure Manual).

9.6.4 The WCSO DNA section has and follows a documented procedure for mixture interpretation that addresses dominant and minor contributors, inclusions and exclusions, and policies for the reporting of results and statistics (refer to the DNA Procedure Manual).

STANDARD 9.7

The policy for the detection and control of contamination is as follows:

9.7.1 A mask must be worn when handling raw evidence of any biological nature.

9.7.2 While examining biological evidence, individuals with long hair must secure hair, e.g. hair clip, rubber band, etc.

9.7.3 Gloves must be changed frequently when processing evidence.

9.7.4 The DNA counters must be cleaned with a bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner, before and after using the work area as well as between each control number during raw evidence exams in the Primary Examination Section.

9.7.5 Sticky mats should be changed frequently.

9.7.6 Appropriate consumables should be cross-linked prior to use.

9.7.7 Anyone (including custodial and maintenance staff) entering the primary exam or pre-amplification DNA laboratories must wear a mask. The doors to these rooms must be maintained in the closed position.

9.7.8 When Primary Examiners and DNA Analysts are working with evidence of a biological nature, they must wear a mask, gloves and a lab coat. It is preferable that each individual keep a pair of dedicated shoes at the laboratory.

9.7.9 Contamination must be investigated and if possible the source of the contamination determined. The Technical Leader will be informed of a contamination event.

9.7.10 Contamination results will be maintained in a contamination log on the Division drive.

9.7.11 See DOC ID's 860 and 5308 for information regarding evidence viewing requirements for individuals observing evidence examination.

STANDARD 9.8

Documentation must be maintained on the following:

9.8.1 Quality control of critical reagents (as defined in 9.3) to include lot and/or batch numbers, and internal evaluations.

9.8.2 The lot number and expiration date of formamide, 3130 Genetic Analyzer 10x Buffer, and POP4 used with the CE 3130 will be recorded in the case packet. It is not necessary to test each new lot of formamide, 3130 buffer, and POP4 before use due to the fact that these chemicals only effect 6% or less of the entire amplified DNA sample at a time and the failure of any of these items is readily detected. The CE 3130 capillary arrays are tracked on the instrument.

STANDARD 9.9

Materials, Reagents, Chemicals and Supplies will be maintained as follows:

9.9.1 Chemicals and reagents should be of suitable quality, correctly prepared, and demonstrated to be compatible with the methods employed.

9.9.2 Formulation: there must be a written procedure for the formulation of reagents, standards, and controls. Each procedure in the DNA Procedure Manual includes the formulation of reagents, standards or controls as appropriate.

9.9.3 Labeling Requirements: all chemicals and reagents must include identity, date of preparation, identity of the individual preparing the reagent, and the expiration date. Where appropriate, special storage requirements should be documented; otherwise all chemicals and reagents are stored according to manufacturer specifications.

9.9.4 Copies of all orders will be maintained by the front office staff in electronic format indefinitely.

9.9.5 Disposal: expired materials, reagents, chemicals, and supplies will not be used in the DNA lab for casework or convicted offender/arrestee analysis. They may be maintained for training and validation purposes or must be disposed of in an appropriate and safe manner. Disposal of hazardous wastes will be handled as described in the SDS's and the Laboratory's Safety Manual.